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(54) Title: IMPLANTABLE AGAROSE-COLLAGEN BEADS CONTAINING CELLS WHICH PRODUCE A DIFFUSIBLE BIOLOGICAL PRODUCT, AND USES THEREOF

(57) Abstract

Implantable beads which are made of agarose and collagen, and/or coated with agarose have incorporated within samples of cells. The cells produce diffusible biological products. The beads may be used as implants to modulate a recipient's immune response. The beads may also be used in an *in vitro* context to encourage specific types of cells to grow, to produce desirable products in culture, or to suppress growth of certain cells. The implants may also suppress growth of certain cells following administration to a subject.

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IMPLANTABLE AGAROSE-COLLAGEN BEADS CONTAINING CELLS WHICH PRODUCE A DIFFUSIBLE BIOLOGICAL PRODUCT, AND USES THEREOF

RELATED APPLICATION

This application is a continuation-in-part of copending application Serial No. 08/625,595, filed April 3, 1996, and incorporated by reference.

Field of the Invention

The present invention relates to the encapsulation of cells in agarose and collagen, followed by coating with agarose, therapeutic methods employing the materials cells, and manufacture thereof.

Background and Prior Art

The encapsulation of various biological materials in biologically compatible materials is a technique that has been used for some time, albeit with limited success. Exemplary of the art are U.S. Patent Nos. 5,227,298 (Weber, et al); 5,053,332 (Cook, et al); 4,997,443 (Walthall, et al); 4,971,833 (Larsson, et al); 4,902,295 (Walthall, et al); 4,798,786 (Tice, et al); 4,673,566 (Goosen, et al); 4,647,536 (Mosbach, et al); 4,409,331 (Lim); 4,392,909 (Lim); 4,352,883 (Lim); and 4,663,286 (Tsang, et al). Also of interest is copending application Serial No. 08/483,738, filed June 7, 1995 to Jain, et al, incorporated by reference herein. Jain, et al discusses, in some detail, the encapsulation of secretory cells in various bio-compatible materials. As discussed therein, secretory cells are cells which secrete biological products. Generally, secretory cells possess at least some properties of endocrine cells, and may generally be treated as equivalent to cells which are endocrine in nature. The copending application discusses, e.g., the encapsulation of insulin producing cells, preferably in the form of islets, into agarose-collagen beads which have also been coated with agarose. The resulting products are useful in treating conditions where a subject needs insulin therapy, such as diabetes.

The Jain, et al application discusses in some detail, the prior approaches taken by the art in transplantation therapy. These are summarized herein as well.

Five major approaches to protecting the transplanted tissue from the host's immune response are known. All involve attempts to isolate the transplanted tissue from the host's immune system. The immunoisolation techniques used to date include: extravascular diffusion chambers, intravascular diffusion chambers, intravascular ultrafiltration chambers, microencapsulation, and macroencapsula-tion. All of these methods have failed, however, due to one or more of the following problems: a host fibrotic response to the implant material, instability of the implant material, limited nutrient diffusion across semi-permeable membranes, secretagogue and product permeability, and diffusion lag-time across semi-permeable membrane barriers.

For example, a microencapsulation procedure for enclosing viable cells, tissues, and other labile membranes within a semipermeable membrane was developed by Lim in 1978. (Lim, Research report to Damon Corporation (1978)). Lim used microcapsules of alginate and poly L-lysine to encapsulate the islets of Langerhans. In 1980, the first successful *in vivo* application of this novel technique in diabetes research was reported (Lim, et al., *Science* 210: 908 (1980)). The implantation of these microencapsulated islets of Langerhans resulted in sustaining a euglycemic state in diabetic animals. Other investigators, however, repeating these experiments, found the alginate to cause a tissue reaction and were unable to reproduce Lim et al's results (Lamberti, et al. *Applied Biochemistry and Biotechnology* 10: 101 (1984); Dupuy, et al., *J. Biomed. Material and Res.* 22: 1061 (1988); Weber, et al., *Transplantation* 49: 396 (1990); and Doon-shiong, et al., *Transplantation Proceedings* 22: 754 (1990)). The water solubility of these polymers is now considered to be responsible for the limited stability and biocompatibility of these microcapsules *in vivo* (Dupuy, et al. *supra*, Weber et al.

supra, Doon-shiong, et al., *supra*, and Smidsrod, *Faraday Discussion of Chemical Society* 57: 263 (1974)).

Recently, Iwata et al., (Iwata, et al. *Jour. Biomedical Material and Res.* 26: 967 (1992)) utilized agarose for microencapsulation of allogeneic pancreatic islets and discovered that it could be used as a medium for the preparation of microbeads. In their study, 1500-2000 islets were micro- encapsulated individually in 5% agarose and implanted into streptozotocin-induced diabetic mice. The graft survived for a long period of time, and the recipients maintained normoglycemia indefinitely.

Their method, however, suffers from a number of drawbacks. It is cumbersome and inaccurate. For example, many beads remain partially coated and several hundred beads of empty agarose form. Additional time is thus required to separate encapsulated islets from empty beads. Moreover, most of the implanted microbeads gather in the pelvic cavity, and a large number of islets are required in completely coated individual beads to achieve normoglycemia. Furthermore, the transplanted beads are difficult to retrieve, tend to be fragile, and will easily release islets upon slight damage.

A macroencapsulation procedure has also been tested. Macrocapsules of various different materials, such as poly-2-hydroxyethyl-methacrylate, polyvinylchloride-c-acrylic acid, and cellulose acetate were made for the immunoisolation of islets of Langerhans. (See Altman, et al., *Diabetes* 35: 625 (1986); Altman, et al., *Transplantation: American Society of Artificial Internal Organs* 30: 382 (1984); Ronel, et al., *Jour. Biomedical Material Research* 17: 855 (1983); Klomp, et al., *Jour. Biomedical Material Research* 17: 865-871 (1983)). In all these studies, only a transitory normalization of glycemia was achieved.

Archer et al., *Journal of Surgical Research* 28: 77 (1980), used acrylic copolymer hollow fibers to temporarily prevent rejection of islet xenografts. They reported long-term survival of dispersed neonatal murine pancreatic grafts in hollow fibers which were transplanted into diabetic

hamsters. Recently Lacy et al., *Science* 254: 1782-1784 (1991) confirmed their results, but found the euglycemic state to be a transient phase. They found that when the islets are injected into the fiber, they aggregate within the hollow tube with resultant necrosis in the central portion of the islet masses. The central necrosis precluded prolongation of the graft. To solve this problem, they used alginate to disperse the islets in the fiber. However, this experiment has not been repeated extensively. Therefore, the membrane's function as an islet transplantation medium in humans is questionable.

Thus, there existed a need for achieving secretory cell transplantation, and, in particular, pancreatic islet allograft and xenograft survival without the use of chronic immunosuppressive agents.

In the Jain, et al work discussed *supra*, the inventors reported that encapsulating secretory cells in a hydrophilic gel material results in a functional, non-immunogenic material, that can be transplanted into animals and can be stored for long lengths of time. The encapsulation of the secretory cells provided a more effective and manageable technique for secretory cell transplantation. The encapsulation technique was described as being useful to encapsulate other biological agents, such as enzymes, micro-organisms, trophic agents including recombinantly produced trophic agents, cytotoxic agents, and chemotherapeutic agents. The encapsulated biological agents were discussed as being useful to treat conditions known to respond to the biological agent.

The application does not discuss at any length the incorporation of cells which produce diffusible biological materials, the latter being useful in a therapeutic context. A distinction is made herein between secretory cells and cells which produce diffusible biological materials. The former, as per the examples given in the Jain application, refers generally to products such as hormones, cell signalling agents, etc., which are normally considered to be biological "messengers". In contrast, diffusible biological materials

refers to materials such as MHC presented peptides, cell expression regulators such as suppressors, promoters, inducers, and so forth. The distinction will be seen in the field of oncology, e.g., as per the following discussion.

Extensive studies in cancer have included work on heterogeneous cell extracts, and various cellular components. Via the use of monoclonal antibodies, the art has identified relevant cancer associated antigens, e.g., GM2, TF, STn, MUC-1, and various epitopes derived therefrom. Current theory postulates that epitopes derived from these various tumor markers complex non-covalently, with MHC molecules, thereby forming an agrotype by specific cytolytic T cells. This mechanism is not unlike various mechanisms involved in the biological response to viral infections. Note in this regard, Van der Bruggen, et al., *Science* 254: 1643-1647 (1991); Boon, et al., 5,405,940, and Boon, et al., 5,342,774, all of which are incorporated by reference.

Additional research which parallels the work on identification of so-called cancer epitopes has focused on the regulation of cancer proliferation, such as via suppression or, more generally, biomodulation. See, e.g., Mitchell, *J. Clin. Pharmacol* 32: 2-9 1992); Maclean, et al., *Can. J. Oncol.* 4: 249-254 (1994). The aim which unites all of these diverse approaches to cancer is the modification of the host's immune response, so as to bring about some improvement in the patient's condition.

Key to all of these approaches is the activity of one or more diffusible biological products which act in concert with other materials to modulate the immune response. Boon, et al. and van der Bruggen, et al., e.g., disclose small peptide molecules. Mitchell discusses larger molecules which function, e.g., as suppressors.

One problem with all therapeutic approaches which employ these materials is the delivery of these in a safe, effective form. This is not easily accomplished. It has now been found, surprisingly, that the techniques of Jain, et al, which were so useful in the development of therapies for conditions

requiring secretory cell products can now be used in other areas.

How this is accomplished is the subject of the invention, the detailed description of which follows.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

This example, and those which follow, employs RENCA cells. These are spontaneous renal adenocarcinoma cells of BALB/C mice, which are widely available, having been maintained in both *in vitro* and *in vivo* cultures. See Franco et al., Cytokine Induced Tumor Immunogenecity, 181-193 (1994).

Samples of frozen RENCA cells were thawed at 37°C, and then placed in tissue culture flasks containing Dulbecco's Modified Medium (D-MEM), which had been supplemented with 10% bovine serum, penicillin (100 u/ml) and streptomycin (50 ug/ml), to give what will be refereed to as "complete medium" hereafter.

Cells were grown to confluency, and then trypsinized, followed by washing with Hank's Balanced Salt Solution, and then with the complete medium referred to *supra*.

In order to determine if the RENCA cells produced tumors efficiently, two BALB/C mice were injected, intraperitoneally, with 10^6 of these cells. The mice were observed, over a 3-4 week period. Clinically, they appeared healthy for the first two weeks, and exhibited normal activity. Thereafter, the clinical manifestations of cancer became evident. One mouse died after 23 days, and the second, after 25 days. Following death, the mice were examined, and numerous tumors of various size were noted. Some of the tumors exhibited hemorrhaging as well.

A sample of one tumor, taken from one of the mice, was fixed in 10% formalin for later histological examination.

Example 2

Following the showing that the RENCA cells did grow *in vivo*, studies were carried out to determine if these cells grew in beads in accordance with the invention.

RENCA cells were grown to confluency, as describ d *supra*, trypsinized, and washed, also as described above. Samples of between 60,000 and 90,000 cells were then prepared. The cells were then centrifuged, at 750 RPMs, and fluid was removed. The cells were then suspended in solutions of 1% atelocollagen, in phosphate buffered saline solution, at a pH of 6.5.

A 1% solution of low viscosity agarose was prepared in minimal essential medium (MEM), maintained at 60°C, and then 100 ul of this were added to the suspension of RENCA cells and atellocollagen, described *supra*. The materials were then transferred, immediately, as a single large droplet, into sterile, room temperature mineral oil. The mixture formed a single, smooth, semi-solid bead. This procedure was repeated to produce a number of beads.

After one minute the beads were transferred to complete medium, as described *supra*, at 37°C. The beads were then washed three times in minimal essential medium containing the antibiotics listed *supra*. The beads were then incubated overnight at 37°C, in a humidified atmosphere of air and 5% CO_2. Following the incubation, the beads, now solid, were transferred to a sterile spoon which contained 1 ml of 5% agarose in minimal essential medium. Beads were rolled in the solution 2-3 times to uniformly coat them with agarose. The beads were transferred to mineral oil before the agarose solidified, to yield a smooth outer surface. After 60 seconds, the beads were washed, five times, with complete medium at 37°C to remove the oil. Overnight incubation (37°C, humidified atmosphere of air, 5% CO_2) followed.

These RENCA containing beads were used in the experiments which follow.

Example 3

Prior to carrying out *in vivo* investigations, it was necessary to determine if the RENCA cells would grow in the beads prepared in the manner described *supra*.

To do this, beads prepared as discussed in example 2 were incubated in the medium described in example 2, for a period

of three weeks under the listed conditions. Three of the beads were then cut into small pieces, and cultured in standard culture flasks, affording direct contact with both the flask and culture medium.

Observation of these cultures indicated that the cells grew and formed standard RENCA colonies. This indicated that the cells had remained viable in the beads.

Example 4

In vivo experiments were then carried out. In these experiments, the beads were incubated for seven days, at 37°C. Subject mice then received bead transplants. To do this, each of four mice received a midline incision, carried through intraperitoneally. Three beads, each of which contained 60,000 RENCA cells were transplanted. Incisions were then closed (two layer closure), using an absorbable suture. The four mice (BALB/C) were normal, male mice, weighing between 24-26 grams, and appeared to be healthy. Two sets of controls were set up. In the first set, two mice received three beads containing no RENCA cells, and in the second, two mice were untreated with anything.

Three weeks after the implantation, all of the mice received intraperitoneal injections of 10^6 RENCA cells. Eighteen days later, one control mouse died. All remaining mice were then sacrificed, and observed.

Control mice showed numerous tumors, while the mice which received the implants of bead-encapsulated cells showed only random nodules throughout the cavity.

These encouraging results suggested the design of the experiments set forth in the following example.

Example 5

In these experiments, established cancers were simulated by injecting RENCA cells under one kidney capsule of each of six BALB/C mice. Fifteen days later, mice were divided into two groups. The three mice in the first group each received three beads, as described in example 4, *supra*. The second group (the control group) r ceived beads which did not contain RENCA cells.

After 4-5 days, mice which had received RENCA cell containing implants looked lethargic, and their fur had become spiky, while the control group remained energetic, with no change in condition of fur.

Ten days after implantation (25 days after injection of RENCA cells), however, the control mice became sluggish, and exhibited distended abdomens. One of the three control mice died at fourteen days following bead transplantation. Sacrifice of the mice followed.

The body cavities of the control mice showed profuse hemorrhaging, with numerous tumors all over the alimentary canal, liver, stomach and lungs. The entire abdominal cavity had become indistinguishable due to rampant tumor growth. The mice which had received beads with encapsulated RENCA cells, however showed no hemorrhaging, and only a few nodules on the alimentary cancers. Comparison of test and control groups showed that, in the test group, nodules had not progressed.

Example 6

Freely inoculated RENCA cell growth is inhibited when incubated along with encapsulated RENCA cells. A further set of experiments were carried out to determine if this effect was observable with other cells.

An adenocarcinoma cell line, i.e., MMT (mouse mammary tumor), was obtained from the American Type Culture Collection. Encapsulated MMT cells were prepared, as described, *supra* with MMT cells, to produce beads containing 120,000 or 240,000 cells per bead. Following preparation of the beads, they were used to determine if they would inhibit proliferation of RENCA cells *in vitro*. Specifically, two, six well petri plates were prepared, via inoculation with $1x10^4$ RENCA cells per well, in 4 ml of medium. In each plate, three wells served as control, and three as test. One of the three control wells in each plate received one bead. Each of the other wells received either two or three empty beads. The second well was treated similarly, with wells receiving one, two or three beads containing 120,000 or 240,000 MMT cells. Wells were incubated at 37°C for one week, after which RENCA

cells were trypsinized, washed, and counted, using a hemocytometer. Results follow:

DISH # 1 (EMPTY MACROBEADS) # of cells retrieved after one week			DISH # 2 (MACROBEADS WITH MMT CELLS) # of cells retrieved after one week	
Well #	Control	Empty	120,000 MMT cells	240,000 MMT cells
1	2.4×10^5	2.8×10^5	1.4×10^5	1×10^5
2	2.0×10^5	3.5×10^5	1.2×10^5	7×10^4
3	4.4×10^5	2.5×10^5	1.25×10^5	9×10^4

Example 7

Following the results in example 6, the same experiments was carried out, using $1x10^4$ MMT cells rather than RENCA cells. The experiment was carried out precisely as example 6. Results are set forth below.

Well #	Control	Empty Macrobeads	(1) MMT Macrobeads	(2) MMT Macrobeads
1	3.1×10^6	2.8×10^6	1.6×10^6	1.3×10^6
2	3.3×10^6	2.6×10^6	1.0×10^6	1.1×10^6
3	3.0×10^6	2.8×10^6	6.0×10^5	5.0×10^5

These results encouraged the use of an *in vivo* experiment. This is presented in example 8.

Example 8

RENCA cells, as used in the preceding examples, are renal cancer cells. To demonstrate more completely the general efficacy of the invention, work was carried out using a different type of cancer cells. Specifically, adenocarcinoma cells were used.

A mouse mammary tumor cell line (MMT) was obtained from the American Type Culture Collection. Using the protocols set forth, *supra*, implants were prepared which contained 120,000 cells per bead, and 240,000 cells per bead.

The experimental model used was the mouse model, *supra*. Twenty two mice were divided into groups of 4, 9 and 9. The first group, i.e., the controls, were further divided into three groups of two, one and one. The first subgroup received implants of one bead containing no cells. One mouse received two empty beads, and one received three empty beads.

Within experimental group A (9 animals), the beads contained 120,000 cells, while in group B, the beads contained 240,000 cells. Within groups "A" and "B", there were three subdivisions, each of which contained three mice. The subgroups received one, two, or three beads containing MMT cells.

Twenty one days following implantation, all animals received injections of 40,000 RENCA cells. Immediately after injection, the mice were lethargic, with spiky hair. This persisted for about five days, after which normal behavior was observed.

After twenty days, control mice exhibited distended abdomens, and extremely spiky hair. One control mouse died 25 days following injection, while the remaining control mice appeared terminal. All mice were sacrificed, and tumor development was observed. These observations are recorded infra:

NUMBER OF MACROBEADS IN MICE	CONTROL	EXPERIMENTAL GROUP A	EXPERIMENTAL GROUP B
1	++++	--	--
1	++++	--	--
1		+	++
2	++++	--	--
2		--	--
2		++	++
3	++++	--	--
3		--	--
3		--	+++

These results show that, of eighteen mice tested, thirteen showed no disease. Of the mice in Group (A), one mouse exhibited a few modules, and another mouse showed a few tumors. One mouse which received two beads showed a few tumors.

Within group B, one mouse which had received one bead, and one mouse which received two beads showed a few tumors, entangled with intestines. One of the mice which received three beads had developed a large solid tumor and was apparently very sick. Nonetheless, the overall results showed that the encapsulated mouse mammary tumor cells inhibited tumor formation.

Example 9

As suggested, *supra*, the practice of the invention results in the production of some material or factor which inhibits and/or prevents tumor cell proliferation. This was explored further in the experiment which follows.

Additional beads were made, as described *supra* in example 2, except that atellocallogen was not included. Hence, these beads are agarose/agarose beads. RENCA cells, as described,

supra, were incorporated into these beads, again as described *supra*.

Two sets of three six well plates were then used as control, and experimental groups. In the control group, wells were filled with 4 ml of RPMI complete medium (10% fetal calf serum and 11 ml/l of penicillin). Each control group well was then inoculated with 10,000 RENCA cells.

In the experimental group, the RPMI complete medium was conditioned, by adding material secured by incubating 10 immuno-isolated, RENCA containing beads (120,000 cell per bead), in a 35x100 mm petri plate containing 50 ml of the RPMI complete medium. Following five days of incubation, medium was collected from these plates, and 4 ml of it was placed in each test well. These wells were then inoculated with 10,000 RENCA cells.

All plates (both control and experimental) were incubated at 37°C for five days. Following the incubation period cells were trypsinization washed, and counted using a hemocytometer. The cells in the plates of each well were pooled together following trypsinization, and counted, the results follow.

WELL #	(CELLS) CONTROL	(CELLS) CONDITIONED
1	7×10^5	3×10^5
2	8×10^5	2.5×10^5
3	7×10^5	3.4×10^5

These results show that the cells, when restricted in, e.g., the beads of the invention, produced some factor which resulted in suppression of tumor cell proliferation. This restriction inhibitor factor is produced by the cells in view of their entrapment in the bead, and differs from other materials such as contact inhibitor factor, which are produced when cells contact each other.

Example 10

The experiment set forth *supra* showed that RENCA cell growth, in conditioned medium, was about half the growth of

the cells in control medium. The experiments set forth herein examined whether the growth inhibiting factor would remain active after the conditioned medium was frozen.

RENCA conditioned medium was prepared by incubating 10 immunoisolated RENCA containing beads for five days. Incubation was in 35x100 mm petri plates, with 50 ml RMPI complete medium, at 37°C. Following the incubation, the medium was collected and stored at -20°C. Conditioned medium was prepared by incubating immunoisolated MMT (mouse mammary tumor) cell containing beads. The beads contained 240,000 cell per bead; otherwise all conditions were the same.

Frozen media were thawed at 37°C, and then used in the following tests. Three six well plates were used for each treatment, i.e., (i) RMPI control medium, (2) RENCA frozen conditioned medium, and (3) MMT frozen conditioned medium. A total of 4 ml of medium was dispensed into each well. All wells were then inoculated with 10,000 RENCA cells, and incubated at 37°C, for five days. Following incubation, two plates of samples were taken from each well, trypsinized, pooled, and counted in a hemocytometer. At eight days, th remaining three plates of each well were tested in the same way.

Results follow:

DISH 5 DAYS OLD	CONTROL MEDIUM	FROZEN CONDITIONED MEDIUM OF RENCA	FROZEN CONDITIONED MEDIUM OF MMT
1	6×10^5	5×10^5	8×10^4
2	6.8×10^5	4.2×10^5	8.5×10^4
8 DAYS OLD			
3	2.8×10^6	2×10^6	8×10^4

When these results are compared to those in example 6, *supra*, it will be seen that, while the frozen/thawed RENCA

conditioned medium did not arrest growth to the same extent that unfrozen medium did (compare examples 6 and 7), it did, nonetheless, arrest growth). Frozen conditioned medium using MMT cells arrested growth even more than the unfrozen MMT conditioned medium. These results show that, of eighteeen mice tested, thirteen showed no disease. Of the mice in Group (A), one mouse exhibited a few modules, and another mouse showed a few tumors.

The foregoing describes the manufacture of implantable beads which contain one or more types of cells which produce a diffusible biological product, as this phrase is defined herein. The diffusible biological product is one which has an effect on the subject in which the bead is implanted. Preferably, this effect is immunomodulation, such as stimulating an immune response, or suppressing a response. In the case of cancer, for example, the diffusible biological product may be a peptide which complexes with MHC molecules on cancer cells in a subject, thereby provoking a CTL response thereto in turn leading to lowering of the tumor load in the subject. The diffusible product may also be a suppressor of tumor growth. In connection with this form of therapy, it is possible, although not necessarily preferable, to place the implanted beads in or near an identified tumor.

"Diffusible biological product" as used herein refers to materials such as proteins, glycoproteins, lipoproteins, carbohydrates, lipids, glycolipids, and peptides. More specifically, materials such as antibodies, cytokines, hormones, enzymes, and so forth, are exemplary, but by no means the only type of materials included. Excluded are the well known "end products" of cellular processes, such as CO_2 and H_2O.

As the experiments show, the implantable beads may also be used prophylactically. It is well known that at least a segment of the population of cancer patients are prone to re-occurrence of the condition. The experiments described herein show that the implants can prevent the occurrence or

reoccurrence of cancer, via the biological effect the diffusible product has on a subject's system.

The discussion of the invention has focused on *in vivo* approaches. It must also be understood that there are *in vitro* approaches to the invention, some of which are discussed upon herein. For example, it is well known that many cells which produce desirable products, when cultured *in vitro*, require the presence of feeder cells. There are always issues with such feeder cells. They may grow faster than the desired cells, leading to de facto "strangulation" of the materials of interest. Further, there can be a problem with various toxic products being produced by the feeder layers. The implantable beads of the invention act almost as cellular incubators, protecting the incorporated cells, while permitting the diffusible products to move into a culture medium, e.g., where they can be collected.

As indicated, *supra*, preparation of the implantable beads first requires suspension of the cells in solution, preferably aqueous of collagen. Preferably, the collagen is atelocollagen, in a solution of from about 0.5 to about 2%. Depending upon the type of cell used, the number of cells in the solution at a given time, and hence the number of cells in a bead, will vary. Preferably, there are from about 10,000 to about 200,000 cells used per bead, more preferably from about 30,000 to about 100,000. Most preferably, about 40,000 to about 60,000 cells are used.

Following suspension of the cells in the collagen solution, an agarose solution is added. Preferably, this agarose solution will range from about 0.5% to about 5%, preferably about 1%. By dropping the mixture onto or into inert materials, such as TEFLON® or mineral oil, a bead forms. This bead is semi-solid. The semi-solid bead is then transferred to a sterile medium, preferably one containing antibiotics, washed, and incubated to polymerize collagen. The polymerization of collagen is a well studied phenomenon, and the conditions under which this occurs need not be elaborated upon herein.

Following the solidification of the bead, it is then coated with agarose, preferably by rolling it in an agarose solution. One preferred way of accomplishing this is a simple TEFLON® coated spoon which contains a solution of agarose, preferably 5% to 10%.

The foregoing discussion of diffusible biological products should not be construed as being limited to wild type materials. For example, one can just as easily incorporate transformed or transfected host cells, such as eukaryotic cells (e.g., 293 cells, CHO cells, COS cells), or even prokaryotic cells (e.g., E. coli), which have been treated to produce heterogeneous protein, or modified via, e.g., homologous recombination, to produce increased amounts of desirable biological products, Other materials, such as hybridomas, may also be used, with the diffusible biological product being a monoclonal antibody.

Other features and aspects of the invention will be clear to the skilled artisan, and need not be related here.

The terms and expression which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expression of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

We claim:

1. A composition of matter comprising an agarose coated, solid agarose collagen bead containing cells which produce a diffusible biological material.
2. The composition of matter of claim 1, wherein said cells are cancer cells.
3. The composition of matter of claim 2, wherein said cancer cells are renal cancer cells.
4. The composition of matter of claim 1, wherein said bead contains from about 10,000 to about 200,000 cells.
5. The composition of matter of claim 4, wherein said bead contains from about 30,000 to about 100,000 cells.
6. Method for administering a diffusible biological material to a subject, comprising implanting the composition of matter of claim 1 in said subject.
7. The method of claim 6, wherein said subject suffers from a pathological condition treatable by said diffusible biological material.
8. The method of claim 7, wherein said pathological condition is characterized by abnormal cell growth.
9. The method of claim 8, wherein said pathological condition is cancer.
10. A process for manufacturing an agarose coated, solid agarose bead which contains cells which produce a diffusible biological material, comprising:

(a) suspending cells which produce said diffusible biological material in a collagen containing material,

(b) adding agarose to said solution,

(c) forming a semi-solid bead of said collagen, agarose, and diffusible biological material produced cells,

(d) polymerizing collagen in said semisolid bead to form a solid, agarose-collagen bead containing said diffusible biological material producing cells, and

(e) coating said solid, agarose-collagen bead containing cells with agarose.

11. The process of claim 10, wherein said cells are cancer cells.

12. The process of claim 10, comprising suspending from about 10,000 to about 200,000 cells in said collagen containing solution.

13. The process of claim 10, comprising suspending from about 30,000 cells to about 100,000 cells in said collagen containing solution.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04548

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 63/00; C12N 5/00, 11/02, 11/10
US CL :424/93.7; 435/177, 178, 382
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.7; 435/174, 177, 178, 180, 182, 382

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, BIOSIS, MEDLINE
search terms: collagen, agarose, beads, cells

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,647,536 A (MOSBACH et al.) 03 March 1987, entire document.	1-13
A	US 4,798,786 A (TICE et al.) 17 January 1989.	1-13
Y	US 4,673,566 A (GOOSEN et al.) 16 January 1987, entire document.	1-13
Y	US 5,053,332 A (COOK et al.) 01 October 1991, entire document.	1-13
Y	US 4,971,833 A (LARSSON et al.) 20 November 1990, entire document.	1-13
Y	US 4,663,286 A (TSANG et al.) 05 May 1987, entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 JUNE 1997	11 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID M. NAFF Telephone No. (703) 308-0196

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